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Biomedical Engineering

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Protein Purification

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Protein Purification

1 Ni^{2+}-affinity chromatography

Estimated bench time: 2 hours
Estimated total time: 2 hours, depending on the amount of samples
Purpose: Purification of protein samples after the Bugbuster cell lysis.

1.1 Materials

- Protein samples
- Charge Buffer (80 mL)
- Wash Buffer (300 mL)
- Elution Buffer (150 mL)
- Strip Buffer (150 mL)
- Columns
- Ni-NTA HisBand resin
- H_2O
- Filter for on top of the resin
- Eppendorf tubes
- TCEP
- Pipette boy

1.2 Setup & Protocol

- Prepare the buffers needed for protein purification:
    - Charge Buffer (80 mL)
      - 50 mM $NiSO_4$
    - Wash Buffer (300 mL)
      - 20 mM Tris-HCL (pH 7.9)
      - 0.5 M NaCl
      - 30 mM imidazole
    - Elution Buffer (150 mL)
      - 20 mM Tris-HCL (pH 7.9)
      - 0.5 M NaCl
      - 500 mM imidazole
    - Strip Buffer (150 mL)
      - 20 mM Tris-HCL (pH 7.9)
      - 0.5 M NaCl
      - 100 mM EDTA
- Load a column with 2-3 mL Ni-NTA HisBind resin (5-10 mg protein per mL resin). Perform the loading by flowing H2O through the column to which resin can be added.
- Add a filter on top of the resin. Make sure that no air gets trapped in the resin or between filter and resin.
- Equilibrate the column with H_2O (~20 mL).
- Add 3 column volumes of Charge Buffer (~8 mL). Collect the waste flow-through separately as this is heavy metal waste (has to be thrown away in specific jerry can in fume hood).

- Add 4 column volumes of Wash Buffer (~12 mL) to remove unbound nickel. Collect the waste flow-through again separately (has to be thrown away in specific jerry can in fume hood).
- Load the protein samples (supernatant from Bugbuster cell lysis) onto the column. Collect a drop of the flow-through for SDS-PAGE analysis.
- Add 7 column volumes of Wash Buffer (~20 mL) supplemented with 1 mM TCEP (prepare in separate Falcon tube) to remove native E. coli proteins that are weakly bound to the column. Collect a drop of the flow-through for SDS-PAGE analysis.
- Elute the protein by adding 4 column volumes of Elution Buffer (~12 mL) supplemented with 1 mM TCEP (prepare in separate Falcon tube). Collect ~0.5 mL elution fractions Eppendorf tubes (the fractions are colorless!). Store a drop of the flow-through for SDS-PAGE analysis.
- Keep the protein elution fractions on ice in a closed bucket. Evt. measure protein concentrations in the elution fractions using the NanoDrop spectrophotometer.
- Strip the Ni^{2+} off the column using 4 column volumes of Strip Buffer (~10 mL). Once more it is necessary to use a separate waste disposal container.
- To preserve the column it is necessary to add a few mL Strip Buffer to the column before sealing.
- Store the column in the fridge at 4 °C.

2 Change of buffer using Amicon Ultra-4 centrifugal filter

Estimated bench time: 1.5 hours
Estimated total time: 2 hours
Purpose: Change of buffer from the protein samples, making sure they are able to be stored at -80 °C.

2.1 Materials

- Protein Storage Buffer (500 mL)
- Centrifugal filters (be careful of the molecular weight!)
- Table-top centrifuge
- Protein samples
- TCEP
- Pipettes
- Eppendorf tubes or Falcon tubes (depending on the amount of sample)
- Liquid nitrogen

2.2 Setup & Protocol

- Prepare the buffer:
    - Protein storage buffer (500 mL)
      - 20 mM HEPES pH 7.5
      - 100 mM NaCl
- Load the protein sample in the centrifugal filter.
- Make sure all filter tubes are well weight-balanced.
- Centrifuge for 30 minutes at 4000 rpm at 4 °C, or longer if the volume of the sample in the filter has not been reduced to ~1:10 of start volume. Discard the flow-through.

- Add buffer (supplemented with 1 mM TCEP) to the sample in the filter till the volume is again 4 mL. Mix the sample with the fresh buffer by pipetting up and down. Never vortex protein samples.
- Centrifuge for 10 minutes at 4000 rpm at 4 °C and repeat the procedure of buffer loading, mixing and centrifugation another 3x (dilute proteins at least ~1000x in new buffer).
- Pipette the protein sample out of the filter into Eppendorf tubes (label these well! Cover labels with normal transparent tape). Mix well and make sure no protein sticks to the membrane of the filter. Keep the protein samples on ice in closed bucket.
- The protein samples need to be frozen via snap freezing in liquid nitrogen. Transfer the samples to the -80 °C freezer.

3 Determine Protein Concentration

Estimated bench time: 15 minutes
Estimated total time: 15 minutes
Purpose: Determining the concentration of the purified protein samples.

3.1 Materials

- Nanodrop Spectrophotometer
- H_2O
- Protein samples

3.2 Setup & Protocol

- Use the Nanodrop spectrophotometer ('UV-Vis').
- First initialize and measure blank with H_2O, and subsequently load 2 µL of protein sample.
- Save the measured UV-Vis spectrum and write down the absorption at 280 nm. Calculate the protein concentration using the extinction coefficient of the protein and the law of Lambert-Beer.
- For later SDS-PAGE analysis of purified protein: dilute protein sample to ~1.5 µM in 10 µL of H_2O.